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Genetic Variation of Yam *Dioscorea* spp. (Family Dioscoreaceae) Accessions of Sri Lanka

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ABSTRACT

The present study determined genetic diversity among seventeen *Dioscorea* accessions comprising eleven *D. alata*, two *D. bulbifera*, two *D. esculenta*, one *D. pentaphylla* and one *D. spicata* conserved at the Plant Genetic Resources Center (PGRC), Sri Lanka. Morphological analysis was done using 55 standard morphological descriptors of Yam and, molecular analysis using 15 SSR markers. Statistical analysis for morphological data was done using MINITAB 16, molecular analysis using PowerMarker version 3.25 and MEGA 6.06 software respectively. Results revealed a significant degree of genetic diversity among tested accessions. Five clusters were observed according to the cluster analysis of morphological characterization. A high variation was observed among *D. pentaphylla* and *D. esculenta* accessions and remaining *Dioscorea* spp. Similarities of morphological characters clustered *D. alata* accessions with *D. bulbifera* and *D. spicata*. Statistically analyzed molecular data revealed a considerable genetic variation among selected accessions generating a total of 55 alleles with polymorphism in 13 SSR markers. Phylogenetic tree constructed based on Nei's (1983) genetic distance and UPGMA algorithm consists of seven major clusters separating *D. alata* onto one cluster and other *Dioscorea* spp. into other different clusters revealing no duplicates among tested accessions.

1. Introduction

Yam (*Dioscorea* L. spp.) are polyploid tuberous monocots belong to the family Dioscoreaceae [1]. Out of the available *Dioscorea* spp. in the world, a few are edible yams that contain a high amount of starch, fiber, and vitamins. The nutrient contents of yam per 100 g fresh edible tuber include 50 - 84% moisture, 15 - 31% carbohydrate, 0.04 - 0.6% crude fat, 1.1 - 2.8% protein and 71 - 142 kcal energy [2, 3]. Easy management, minimum pest, and disease attacks, lower input requirement, ease of cultivation as well as high medicinal value contributes for the high demand for *Dioscorea* spp. [4].

Yam is one of the seasonal crops cultivated in Sri Lanka. Most popular *Dioscorea* spp. in Sri Lanka are *Dioscorea alata* or 'Mahavelala' and *D. esculenta* (Lesser yam) while *D. bulbifera* is consumed at a lesser extent [5]. These are also recommended varieties by the Department of Agriculture, Sri Lanka to cultivate in all three climatic zones in the country [6].

Genetic diversity includes the differences in inheritable characteristics in a population of the same species. This enables different organisms to adapt to different environmental conditions [7]. Thousands of valuable allelic variations of traits of economic significance remain unutilized in nearly all the crop plants [8]. Discovery of the genetic variability of the crop plants can be an answer to the world food crisis and also can be effectively incorporated in crop improvement programs as well [9].

Characterization and evaluation make the proper identification of germplasm, which is the first step in utilization. Molecular markers can support the detailed and reliable characterization of genetic

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resources. Identification of *Dioscorea* sp. is complicated by the fact that farmers with different ethnicity have different vernacular names possibly for the same genotypes leading to confusion in the number of varieties of available yam germplasm [1].

Therefore morphological and molecular characterization of 17 selected accessions of *Dioscorea* spp. was carried to assess the genetic diversity.

2. Material and Methods

Morphological and molecular characterization of 17 selected *Dioscorea* accessions.

Table 1. Accessions used for the study.

Species	Accessions
D. alata	A1 – A 11
D. esculenta	E1, E2
D. bulbifera	B1,B2
D. pentaphylla	P1
D. spicata	S1

2.1. Morphological analysis

Morphological data were collected from five selected one month old plants in each accession using standard descriptors for Yam published by the IPGRI/IITA, 1997 [10]. A total of 55 morphological characters were scored for the selected accessions and were analyzed using Minitab 16 software.

Table 2. Morphological characters used for the study

Part of the plant	Character
Young stem	1. Young stem color 2. Absence/presence of waxiness 3. absence/presence of wings 4. wing color 5. absence/presence of hairs 6. absence/presence of spines 7. absence/presence of barky patches 8. Stem vigor 9. twining habit 10. twining direction 11. no of stems per plant
Mature stem	12. mature stem color 13. stem diameter 14. internode length 15. absence/presence of waxiness 16. absence/presence of wings 17. wing position 18. wing size 19. wing color 20. hairiness 21. absence/presence of wrinkled surface 22. spines on stem base 23. spines on stem above 24. spine position 25. spine shape 26. spine length
Young leaf	27. young leaf color 28. leaf margin color 29. vein color 30. petiole color 31. petiole wing color
Mature leaf	32. position of mature leaves 33. leaf density 34. internodes to fully expanded leaf 35. leaf type 36. leaf lobation 37. number of leaflets in compound leaf 38. leatheriness 39. mature leaf color 40. leaf vein color 41. lower surface vein color 42. leaf margin color 43. hairiness of upper surface 44. hairiness of lower surface 45. waxiness of upper surface 46. leaf shape 47. undulations of leaf 48. distance between lobes 49. downward arching along main veins 50. upward folding to form a cup 51. position of widest part of leaf 52. leaf length 53. tip length 54. tip color 55. petiole length

2.2. Molecular analysis

Fifteen *Dioscorea* spp. accessions were characterized using 15 SSR markers.

2.2.1. Extraction of genomic DNA

DNA extraction protocol [11] was optimized for both immature and mature leaves of *Dioscorea* spp. Leaves of approximate weight of 1.00 g were crushed using liquid nitrogen. The ground material was suspended in 800 µL of pre-warmed (65ºC) Isolation buffer (100 mMTris-HCl (pH 8.0), 50 mMEDTA (pH 8.0) in a 1.5 mL microcentrifuge tube containing in a water bath, β - mercaptoethanol was added in a final concentration of 2% to prevent oxidation of polyphenols .about 100 µL 10% SDS was also used as a detergent. All were mixed thoroughly, vortexed and incubated in a shaker, at 65 ºC for 30 minutes. Then 350 µL of 5 M potassium acetate was added and mixed vigorously. It was cooled on ice for 5 minutes and then centrifuged at

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10,000 rpm for 25 minutes at 4 ºC temperature using a centrifuge. The supernatant was transferred into a clean micro centrifuge tube. About 535 µL of cold isopropanol was added and mixed gently to precipitate the DNA. The tubes were then inverted gently till DNA is precipitated. Next, the tubes were stored at -20 ºC for overnight to increase the yield of nucleic acid. DNA was spooled out. If not, centrifuged at 10,000 rpm for 10 minutes at 4ºC and Supernatant was decanted. DNA pellet was rinsed with 500 µL of cold 70% ethanol. Then ethanol was completely drained and pellet was dried several hours in a fume hood until all traces of ethanol are completely removed. About 120 µL of dissolution buffer (50 mMTrisHCl of pH 8, 10 mM EDTA) was added to the DNA pellet to dissolve DNA. To dislodge the pellet, it was tapped gently and incubated at 55 ºC for 10 minutes in a water bath. The solution was mixed gently and cooled on ice for 2 minutes. Then, it was centrifuged at 10,000 rpm for 5 minutes at 4 ºC to remove any undissolved material. The supernatant was transferred to a clean micro centrifuge tube and 12 µL of 3 M sodium acetate solution (pH 5.2) and 88 µL of cold isopropanol was added to re-precipitate DNA. Then DNA was spooled out or if not centrifuged at 10,000 rpm for 5 minutes at 4 ºC. The supernatant was decanted and pellet was washed with 500 µL of cold 70% ethanol. Then ethanol was completely drained and pellet was dried several hours in a fume hood until all traces of ethanol are completely removed. Finally the pellet was dissolved in 50 µL of TE buffer (1 M Tris - HCl of pH 8, 0.5 M EDTA of pH 8.0). Afterwards they were stored at -20ºC until further use.

2.2.2. DNA confirmation, quantification and dilution

DNA were run in 0.8% Agarose Gel Electrophoresis (60 V, 1 hour) for the DNA confirmation, quantification and dilution. The gel was stained in 0.5 µL/mL Ethidium bromide and observed under UV light in Bio-Rad Gel Documentation System. DNA concentration was quantified using standard DNA ladders. DNA samples were diluted to about 15 ng/µL with TE buffer and about 3 µL volume was loaded from each DNA sample.

2.2.3. Polymerase chain reaction (PCR)

PCR was done for 15 simple sequence repeat (SSR) primers (Table 1) based on sequence information given by [12] and [13]. PCR protocol was optimized using temperature gradients and a 10 µL of PCR cocktail mixture was composed of 2 µL of 5x PCR buffer, 0.25 µL of 10mm dNTP, 0.68 µL of 2.5Mm of MgCl2, 0.2 µL of 20 µM forward and reverse primer, 0.13 µL of 5 units/µL Taq, 2.5 µL of DNA template of 10 ng/µL and 4.04 µL of sterile distilled water [3].

PCR amplification was done for 15 SSR markers namely, Da1F08, Dab2C05, Dab2D08, Dpr3B12, Da1A01, YM13, Da1C12, Dab2E07, C5, F1, H12, H2, mDaCIR20, mDaCIR55, mDaCIR17 [3, 11, 12]. PCR program consisted of an initial denaturation step at 94 ºC for 4 minutes followed by 35 cycles consisted of 3 steps namely, a denaturation step at 94 ºC for 30 seconds, annealing step for 1 minute in which the specific temperature for each primer was maintained and an extension step at 72 ºC for 1 minute. At the end of the final cycle, final extension was carried out at a temperature of 72 ºC for 7 minutes, with subsequent holding temperature at 4 ºC. Total time for PCR program was 1 hour and 58 minutes.

2.2.4. PCR confirmation and Polyacrylamide gel electrophoresis

PCR products were detected using 1.5% Agarose gel electrophoresis and bands were visualized using 8% non-denaturing Polyacrylamide Gel Electrophoresis (PAGE) in 1X TBE buffer (Pre-run at 200 V, 36-60 mA for about 1 hour. Gels were stained in 0.5 µg/mL Ethidium bromide and then DNA bands were visualized using Bio-Rad Gel documentation system.

2.2.5. Data scoring and analysis

DNA bands in the PAGE image were scored manually using Quantity one version 4.6.3 software. Data analysis was done using PowerMarker version 3.25. The genetic parameters included Nei's gene diversity and polymorphism information content (PIC). Cluster analysis was performed to construct dendrogram based on the similarity matrix data using the unweight pair group method (UPGMA). MEGA 6.06 was used to build the dendrogram.

3. Results and Discussion

3.1. Morphological analysis

The results showed a considerable level of variability among the studied accessions. Most accessions had purple color young stem and few had a green or brownish green color stems. About 54% of the evaluated accessions had a winged stem while the remaining plants were having non-winged stems. Twining direction was anti clockwise for majority of plants. More than 64% of plants were having purple color young leaf where 29% had pale green and 5% yellowish young leaves. Out of the seventeen, three accessions had green colored petioles with purple color at base, five accessions which is 29.41% was with a Green color petiole and remaining 52.94% accessions had a green color petiole with purple

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color at both ends. Evaluation identified 4 distinct leaf shapes. Majority of them were showing cordate leaf shape and others showed saggitate – long, cordate –long and ovate leaf shapes respectively.

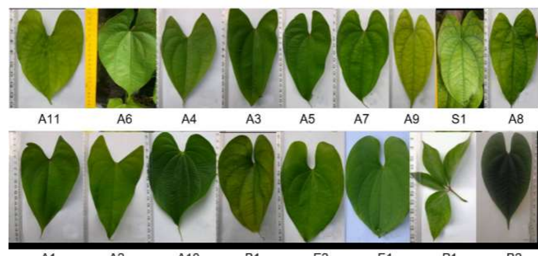


Figure1. Differences among leaf shapes used for the study.

Out of the quantitative characters Number of stems per plant and number of Internodes to fully expanded leaf didn't show a higher variation. Stem girth and internode length of the wine showed a higher variation ranging from 0.5- 1.0 cm and 3.0-9.0 cm respectively.

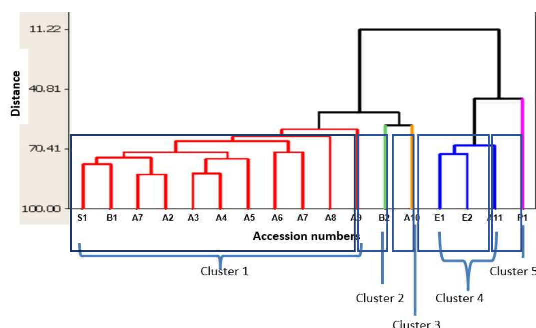


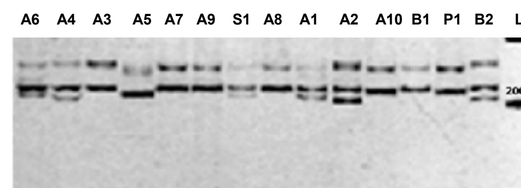
Figure 2. Dendrogram of Yam accessions based on morphological characters

The analysis has assigned the selected accession into 5 main clusters with more than 60% similarity. The cluster tree was separated at 11.22 into two groups; the first group separated into two subgroups nearly at 55; the first subgroup contains all the *D. alata* accessions except A10 due to the large number of similarities in young leaves and stem. Also *D. spicata* and *D. bulbifera* accessions were clustered together in it. This observation might be due to the fact that identical materials may have different names in different collections and areas due to numerous vernacular names. The second subgroup contains only one *D. bulbifera* accession. The large second cluster separates into two nearly at 42 including two subgroups. The first subgroup includes *D. esculenta* accessions with one *D.alata* accession with a number of similarities, while the second subgroup includes the only *D. pentaphylla* accession as the only accession with a compound leaf among studied accessions.

3.2. Molecular analysis

A considerable degree of genetic diversity was exhibited in the molecular analysis among the tested accessions based on SSR polymorphism. From the fifteen SSR markers used in the study thirteen were polymorphic among the tested accessions.

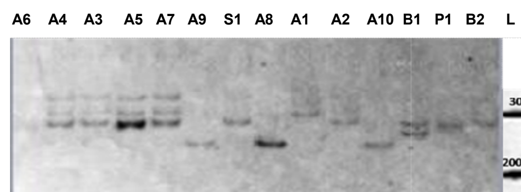
A6 A4 A3 A5 A7 A9 S1 A8 A1 A2 A10 B1 P1 B2 L



Product size- 150-200 Lane: L- 15-200kb ladder

Figure 3.Ethidium Bromide Stained Polyacrylamide Gel Images of PCR Products of the Primer Da1A01

A6 A4 A3 A5 A7 A9 S1 A8 A1 A2 A10 B1 P1 B2 L



Product size- 200-300 Lane: L- 15-200kb ladder

Figure 4. Ethidium Bromide Stained Polyacrylamide Gel Images of PCR Products of the Primer Dab2E07

The number of alleles detected for all loci ranged from 1 to 2 deducing low levels of polymorphism of alleles.

3.3. Cluster analysis based on SSR marker analysis

The genotypic data obtained for well amplified 14 *Dioscorea* spp. accessions by PCR with different primers were analyzed using PowerMarker version 3.25 software and Nei's 1983 frequency based distance matrix. A dendrogram was obtained using Mega 6.06 software. The SSR primer data and Nei 1983's frequency based distance matrix are stated in table 2 and figure 3, respectively.

Minimum genetic distance was observed between accession A3 and A4 which was categorized under *D. alata* and the maximum distance was observed between the accession A1 which was categorized as *D. alata* and accession B1 which was categorized under *D. bulbifera*. The highest PIC value was observed for the marker YM13 (0.77) and the lowest (0.51) for the marker C5 with the average PIC value of 0.68. All SSR markers had PIC value greater than 0.5 and thus were informative for differentiating among the *Dioscorea* accessions used for the study.

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The dissemination of the allele frequency at a single locus differs among the genotypes [14]. The allele frequency revealed by SSR markers for *Dioscorea* accessions was quite low when compared with many other species such as soybean [15]. Genetic diversity displayed the possibility of two randomly chosen alleles to differ from the population. The genetic diversity values ranged from 0.41 to 0.80 with an average of 0.64 (Table 3). The utilization of SSR marker might be a pertinent factor in assessing genetic diversity.

Table 3. Allele number, genetic diversity and PIC values for the SSR primers obtained from PowerMarker V3.25

Marker	Allele No	Gene Diversity	PIC
DA1A01	5.00	0.65	0.60
Dab2D08	3.00	0.56	0.57
Dab2E07	5.00	0.75	0.70
Dab2C05	3.00	0.56	0.57
Da1C12	5.00	0.70	0.66
YM13	6.00	0.80	0.77
Da1F08	5.00	0.63	0.59
mDaCIR17	3.00	0.56	0.50
H2	4.00	0.58	0.52
C5	2.00	0.41	0.51
mDaCIR20	5.00	0.77	0.73
H12	5.00	0.73	0.68
mDaCIR55	4.00	0.66	0.61
Mean	4.23	0.64	0.68

Source: Powermarker V3.25

The results were analyzed to obtain the dendrogram which is shown in figure 5.

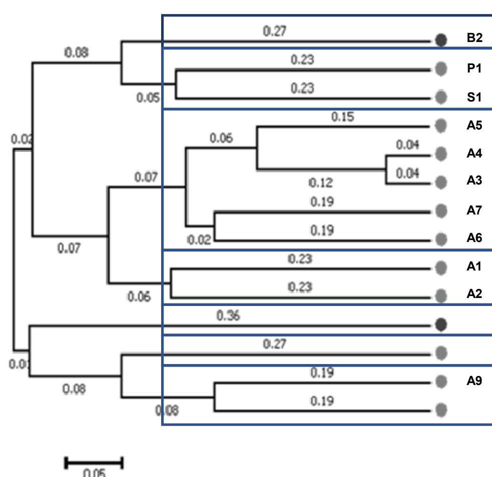

Figure 5. Dendrogram for the studied *Dioscorea* accessions

The cluster tree was separated into two groups; the first group separated into two subgroups nearly; the first subgroup contains a *D. bulbifera* accession. The second subgroup contains *D. pentaphylla* and *D. spicata* accession. All the accessions included in the second sub group belong to *D. alata* separating into two mini groups. The large second cluster separates into two nearly including three subgroups. The first subgroup includes a *D. bulbifera* accession second, *D. alata* accession and the third sub group was composed of two *D. alata* accessions.

In the present study, a significant degree of genetic diversity was found among the studied accessions. Morphological and molecular characterization using stem and leaf characters effectively delineated the seventeen and fifteen accessions respectively. The DNA markers and morphological traits generally do not get closely matching results [16, 17]. Morphological characters can be used to identify some of the accessions to a certain extent as it revealed how distantly some accessions were related even though they were categorized into the same species.

The clustering pattern of *D. alata* accessions A2 – A7 were more or less similar in both morphological and molecular analysis. Further *D. bulbifera* accession B1 and *D. pentaphylla* accession P1 were clustered distantly from other groups.

Accession A11 was categorized under *D. alata* was showing similar morphological characters to *D. esculenta* accessions E1 and E2 in all mature leaf and stem characteristics. In the molecular level they were not amplifying bands with any of the used SSR markers. Therefore they should be investigated further.

4. Conclusion

No duplicates were identified among the selected accessions. Thus, it can be concluded that both morphological and molecular markers could be efficiently used to study genetic diversity in *Dioscorea* genotypes. Even though morphological characteristics could be affected by the environment, their results could be supported by the molecular markers results.

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References

1. Norman PE. Morphological and cytological diversity of some yams (*Dioscorea* spp.) in Sierra Leone, M.Sc. Thesis, University of KwaZulu-Natal, South Africa; 2010. 234p.
2. Asiedu R, Wanyera N M, Nig SY, Nig NQ. Biodiversity in trust: Conversation and use of plant genetic resources in CGAIR Centres. Cambridge University Press, Cambridge, UK; 1997. 57p.
3. Opara LU. Yam storage. In: CGIAR Handbook of Agricultural Engineering Volume IV Agro Processing. Bakker-Akkema (ed.). The American Society of Agricultural Engineers St. Joseph, M.I, USA;1999. 182p
4. Anokye M, Tetteh JP, Otoo E. Morphological Characterization of Some Water Yam (*Dioscorea alata* L.) Germplasm in Ghana. Journal of Agricultural Science and Technology; 2014. 518p.
5. Senanayake SA, Ranaweera, KKDS, Bamunuarachchi A and Gunarathne A. Proximate Analysis and Phytochemical and Mineral Constituents in Four Cultivars of Yams and Tuber Crops in Sri Lanka. Tropical Agricultural Research & Extension; 2012. p15.
6. Ray RC and Sivakumar PS. Traditional and novel fermented foods and beverages from tropical root and tuber crops: review. Int. J. Food Sci. & Tech; 2009. 44, 1073p.
7. Mignouna HD, Abang MM, Asiedu R. Genomics of yams, a common source of food and medicine in the tropics. In P. H. Moore and R. Ming (Eds.), Genomics of tropical crop plants; 2008. 549p.
8. Dumont R, Dansi A, Vernie P, Zoundjihékpon J. Biodiversity and domestication of traditional cultivar *Dioscorea rotundata*. IPGRI, France; 2005. 57p
9. IPGRI/IITA. Descriptors for Yam (*Dioscorea* spp.). Ibadan: International Institute of Tropical Agriculture; 1997.
10. Tostain S, Agbangla C, Scarcelli N. Genetic diversity analysis of Yam cultivars (*Dioscorea rotundata*) in Benin using sequence repeated (SSR) markers. Plant genetic resources. Characterization and utilization; 2007. 71p.
11. Asemota HN. A Fast, Simple, and Efficient Miniscale Method for the Preparation of DNA from Tissues of Yam (*Dioscorea* spp.). Plant Molecular Biology Reporter;1995; 214p.
12. Ooto E, Akromah R, Kolesnikova-Allen M, Asiedu R. Delinearation of pona complex of yam in Ghana using SSR markers. International Journal of Genetics and Molecular Biology; 2009. 006p
13. Siqueira MVBM, Marconi TG, Bonatelli ML, Zucchi MI, Veasey EA. Development and characterization of microsatellite markers in water yam (*Dioscorea alata*). American Journal of Botany; 2011.142p
14. Amelework B, Shimelis H, Tongoona P, Laing M, and Mengistu F, “Genetic variation in lowland sorghum (Sorghum bicolor (L.) Moench) landraces assessed by simple sequence repeats,” Plant Genetic Resources; 2015.
15. Bisen A, Khare D, Nair P, and Tripathi V, “SSR analysis of 38 genotypes of soybean (Glycine Max (L.) Merr.) genetic diversity in India,” Physiology and Molecular Biology of Plants; 2014.
16. Martinez L, Cavagnaro P, Masuelli R. Evaluation of diversity among Argentine grapevine (Vitis vinifera L.) varieties using morphological data and AFLP markers; 2005. 37p.
17. Vollmann J, Grausgruber H, Stift G, Dryzhyruk V, Lelley T. Genetic diversity in camelina germplasm as revealed by seed quality characteristics and RAPD polymorphism. Plant Breeding; 2005. 446p.

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